MODEL CLAMP: A COMPUTER TOOL TO PROBE ACTION POTENTIAL TRANSFER BETWEEN CARDIAC CELLS

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Abstract – **In the early nineties, Joyner and coworkers introduced the "coupling clamp" technique in which an isolated cardiac cell can be electrically coupled to either another isolated cardiac cell or to an analog model cell (RC circuit). In brief, an amplifier system does a continuous analog computation of the current that would be flowing between the two cells if there had been an intercellular coupling conductance G_c, and then provides current inputs to the cells accordingly. Building on this concept, we developed the computer-controlled "model clamp" technique, in which an isolated cardiac cell is dynamically coupled in real time to a comprehensive mathematical cell model (e.g., the phase-2 Luo-Rudy model). With this system we have the ability to vary coupling conductance, effective size of both model cell and real cell, and intrinsic cellular properties of the model cell. In courses on cardiac electrophysiology, the model clamp system provides a useful computer tool to probe action potential transfer between cardiac cells. It can be used to assess alterations in the critical value of coupling conductance required for action potential transfer from a real ventricular cell to the Luo-Rudy model ventricular cell upon exposure of the real cell to, e.g., noradrenaline.**

Keywords – **Heart, electrophysiology, ventricular cells, action potentials, gap junctions, computer simulations**

I. INTRODUCTION

Cardiac activation patterns depend on the multidimensional distribution of cellular membrane properties and intercellular electrical coupling. The anatomical complexity of cardiac tissue, however, makes it difficult, if not impossible, to use tissue experiments to investigate how cellular properties and intercellular coupling influence the observed experimental behavior. Several experimental approaches have been made to study electrical interactions between cardiac cells as a function of intercellular conductance without the complexity of a multidimensional syncytium. In the early nineties, Joyner and coworkers introduced the "coupling clamp" system in which two isolated cells not in physical contact with each other can be electrically coupled at any desired value of intercellular conductance by means of an external circuit that continuously applies time-varying currents to each cell with a sign and magnitude that would have been present if the cells would have been physically coupled. The coupling clamp system allows the rapid independent measurement of the intrinsic cellular properties and then the analysis of the effects of a wide range of intercellular conductance values on the electrical behavior of the cells. At any time during the experiment, the measurements of intrinsic cellular properties can be repeated by temporarily uncoupling the cells.

In their initial paper [1], Joyner and coworkers documented this "coupling clamp" technique and showed that coupling an isolated rabbit ventricular cell to a passive resistance-and-capacitance (RC) circuit representing an inexcitable cell with a normal resting potential, produced a progressive shortening of the action potential duration of the cell as coupling conductance was increased with minimal changes in excitability of the cell. In subsequent studies, the coupling clamp technique was used to study the effect of "injury current" on an isolated rabbit ventricular cell by coupling the cell to a depolarized RC circuit [2], unidirectional block between two isolated rabbit ventricular cells [3], modulation of action potential conduction between two isolated guinea pig ventricular cells by calcium current [4], production of early afterdepolarizations in an isolated guinea pig ventricular cell coupled to a depolarized RC circuit [5], and calcium currents of ventricular cell pairs during action potential conduction [6]. In neuroscience, a similar electronic circuit has been used to create an artificial electrical synapse [7]. However, Scott [8] was probably the first to develop an "Ersatz Nexus," which he used to study the interaction of two aggregates of cultured embryonic chick heart cells with different intrinsic beating rates.

Building on this concept, we suggested the development of a computer-controlled coupling clamp system, as well as a "model clamp" system, in which an isolated cardiac cell is dynamically coupled in real time to a comprehensive mathematical cell model [9]. In 1996, the first two papers on this "model clamp" system were published [10, 11]. We had implemented the model clamp technique with our previously published model of an isolated rabbit sinoatrial node cell [12] ("SAN model clamp") and with the phase-2 Luo-Rudy (LR) model of a guinea pig ventricular cell [13] ("LR model clamp"). In our initial experiments, the SAN model clamp system was used to study synchronization of sinoatrial node cells [10], whereas the LR model clamp system was used to investigate the effects of geometrical asymmetry on action potential conduction between ventricular cells [11]. In subsequent studies, we used the SAN model clamp system with ventricular cells as an experimental model for an ectopic focus [14, 15], and with atrial cells to study atrial activation [16], whereas the LR model clamp system was used with guinea pig ventricular cells to evaluate the effects on action potential conduction of modulation of L-type calcium current [17] or exposure to an 'ischemic' solution [18]. Next, we ex-

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tended the LR model clamp system to include the real-time simulation of a linear strand or a two-dimensional array of LR model cells [19–21]. Another extension of the LR model clamp system is the simultaneous measurement of calcium transients in the real cell using a confocal laser scanning microscope [22]. We used the digital coupling clamp technique to further study the synchronization of sinoatrial node cells [23] and the facilitation of discontinuous action potential propagation between atrial cells by fast pacing [24]. Recently, as presented by Dr. Verkerk in another session at this meeting, we used our model clamp technique to study action potential transfer at the Purkinje-ventricular junction [25].

Other investigators have used an analog coupling clamp circuit in studies of interactions between Purkinje and ventricular myocytes [26–28], beat-to-beat repolarization variability in ventricular myocytes [29], and effects of transient outward current inhibition on conduction between ventricular myocytes [30]. Spitzer et al. [31] used the analog coupling clamp technique to study electrotonic effects of electrically coupling atrioventricular nodal cells to each other and to real atrial or ventricular cells or passive models of such cells (RC circuits). Watanabe et al. [32] studied electrotonic modulation of sinoatrial node pacemaker activity by atrial muscle by coupling together sinoatrial node cells and an RC circuit as a model of an atrial cell. We have recently used the analog coupling clamp ourselves to study how injury current modulates afterdepolarizations in single human ventricular cells [33] and to study the effects of cell-to-cell uncoupling on sheep Purkinje and ventricular action potentials [34].

In this short paper, we first give a detailed description of the coupling clamp and model clamp techniques. Next, we show some results we obtained when coupling together a real guinea pig ventricular cell and the LR model ventricular cell.

II. Methodology

A. Analog Coupling Clamp

With the analog coupling clamp technique an isolated cardiac cell can be electrically coupled to either another isolated cardiac cell or to an analog model cell. In brief, an amplifier system does a continuous analog computation of the current that would be flowing into or out of each cell if there had been an intercellular coupling conductance G_c (siemens) between the two cells. If V_1 is the time-varying membrane potential (volts) of cell 1 and V_2 is the time-varying membrane potential of cell 2, then there would be a time-varying coupling current I_c (amperes) flowing from cell 1 to cell 2 (positive or negative) given by $I_c = G_c \times (V_1 - V_2)$. Two amplifiers continuously compute $V_2 - V_1$ and $V_1 - V_2$, respectively. The outputs of these amplifiers go through V-to-I converters and then back to the cells to provide the current inputs to cell 1 and cell 2 that would have been conducted through a real intercellular conductance G_c (Fig. 1A). The specification of the value of G_c is a combination of the fixed gain of the V-to-I converters and the variable gain of the two amplifiers. An analog cell model, e.g., an RC circuit, may be substituted for one of the cells and connected to the headstage of the dual amplifier.

B. Digital Coupling Clamp

As an expansion of the analog coupling clamp technique we have developed a "digital coupling clamp" system that can provide a variable effective coupling conductance between two single isolated cardiac cells which are not actually in direct contact with each other (Fig. 1B). In brief, this system consists of custom-written software running on a fast micro-

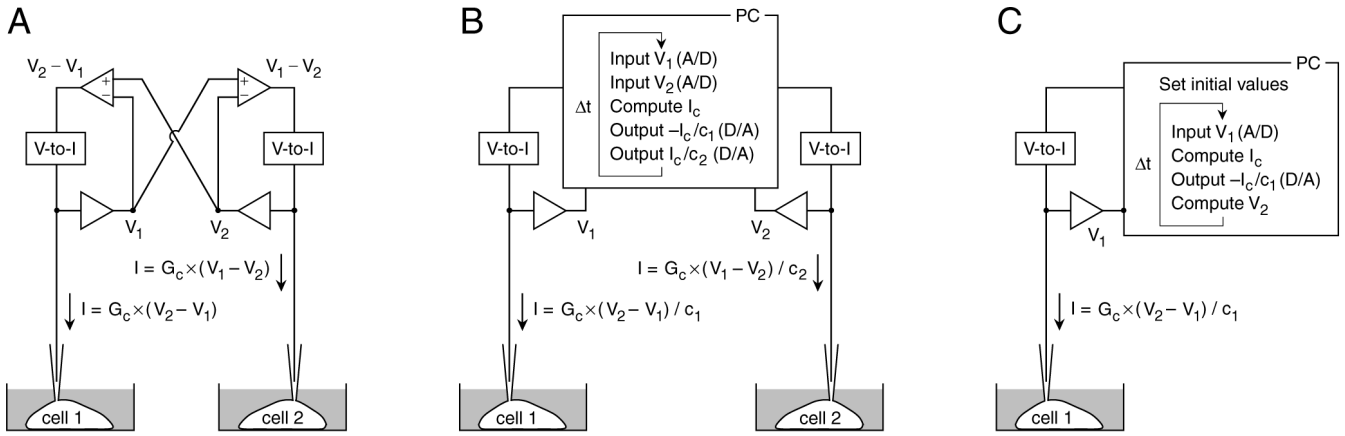

Fig. 1. Experimental techniques to study how two cells not physically connected interact with each other when they are electrically coupled with a variable coupling conductance G_c. (A) Analog "coupling clamp" technique. Membrane potentials V_1 and V_2 of two isolated cells, cell 1 and cell 2, respectively, are recorded using a dual amplifier in the current clamp mode. Two additional amplifiers continuously compute $V_2 - V_1$ and $V_1 - V_2$, respectively, using an effective gain such that the voltage-to-current (V-to-I) converters continuously supply currents $G_c \times (V_2 - V_1)$ and $G_c \times (V_1 - V_2)$ to cell 1 and cell 2, respectively, i.e., the currents that would have been conducted through a real intercellular conductance G_c. (B) Digital coupling clamp technique. Membrane potentials V_1 and V_2 of two isolated cells, cell 1 and cell 2, respectively, are recorded using a dual amplifier in the current clamp mode, and sampled into a microcomputer (PC). The coupling current flowing from cell 1 to cell 2, I_c, is computed according to $I_c = G_c \times (V_1 - V_2)$, and command voltages for the V-to-I converters are generated such that these supply currents $G_c \times (V_2 - V_1) / c_1$ and $G_c \times (V_1 - V_2) / c_2$ to cell 1 and cell 2, respectively, where cell 1 and cell 2 have effective sizes c_1 and c_2 times their actual sizes, respectively. (C) "Model clamp" technique. Cell 2 of panel B has been replaced with a mathematical model cell. The membrane potential of the real cell, V_1, is recorded using a dual amplifier in the current clamp mode, and sampled into a microcomputer (PC). The coupling current, I_c, is computed and a command voltage is generated such that a current input $-I_c/c_1$ is supplied to the real cell, cell 1. The membrane potential of the model cell, V_2, is computed from the mathematical model with the current input for this cell, I_c/c_2, as an additional ionic current.

computer equipped with a fast data acquisition board. When coupling two single isolated cells, both V_1 and V_2 are fed into the computer through the A/D converter of the data acquisition board. Next, I_c is computed and command voltages proportional to I_c (positive or negative) are generated through the D/A converters of the data acquisition board and fed into the *V*-to-*I* converters of the clamp amplifiers to provide the current inputs to cell 1 and cell 2 that would have been conducted through a real intercellular conductance G_c (Fig. 1B). The system allows the effective size of cell 1 (cell 2) to be changed by a factor c_1 (c_2) by replacing the current input for this cell, $-I_c$ (I_c), with $-I_c/c_1$ (I_c/c_2). Thus, the effective size of either cell can, e.g., be doubled by halving its current input.

C. Model Clamp

Replacing real cell 2 in the digital coupling clamp system of Fig. 1B with a mathematical model of this cell, we obtain the "model clamp" system of Fig. 1C. In this system, the membrane potential of cell 2 is not recorded from a real cell, but computed from a detailed mathematical model of an isolated cell. A current input $-I_c/c_1$ is supplied dynamically to the real cell, cell 1, to produce the effect of the (mutual) interaction with the model cell, cell 2, whereas the membrane potential of the model cell, V_2, is computed with the current input for this cell, I_c/c_2, as an additional ionic current to produce the effect of the (mutual) interaction with the real cell.

In our initial implementation, all software was compiled as a DOS real mode application using Borland Pascal 7.0. For numerical integration of differential equations we applied a simple and efficient Euler scheme with a fixed time step, Δt. To speed up calculations in the real-time solution of the single cell model, we fixed the intracellular sodium and potassium ion concentrations, which do not change noticeably during the course of an action potential, and used table look-ups of several model variables, e.g., fully-activated currents, and steady-state values and time constants of gating variables. Also, all computations were performed using the 4-byte "single" variable format of the computer's floating point unit, i.e., with a degree of precision of 7–8 decimal figures, instead of the 10-byte "extended" format. Using a 60-MHz Pentium processor computer and a moderately fast A/D-board (Advantech PCL-718), a time step, Δt, of 130 μs could be achieved for the SAN model clamp system. Using a 90-MHz Pentium processor computer and a significantly faster A/D-board (Axon Instruments DigiData 1200), the LR model clamp system could be run with a time step of 70 μs. Today, with increased processor speeds, much shorter time steps are possible. At the same time, the number of model cells can be increased to simulate strands or sheets of cells [19–21, 25].

III. Results

The model clamp system may be used to demonstrate the role of L-type calcium current ($I_{Ca,L}$) in discontinuous conduction. This is illustrated in Fig. 2, which shows results of a study in which we pharmacologically modulated $I_{Ca,L}$ of an isolated guinea pig ventricular cell that was the leader (stimulated) cell of the hybrid cell pair [17]. The follower cell was the LR model cell. The size of the real cell was adjusted through the factor c_1 (Fig. 1) to have the same current threshold for a 2-ms pulse as the LR model (2.6 nA). With the intercellular coupling conductance G_c set to 6.6 nS, the conduction delay was about 20 ms in control solution. Reducing $I_{Ca,L}$ by application of nifedipine (to the real cell) produced conduction failure at this value of G_c, while for the same cell application of isoproterenol, enhancing $I_{Ca,L}$, shortened the conduction delay. Both effects were completely reversible. The critical value of G_c for successful conduction was 6.8±0.1 nS (mean±SEM, n=17) in control solution. Critical G_c was significantly decreased by 20 nM isoproterenol (5.3±0.2 nS, n=8, p<0.001) and increased by 1 μM nifedipine (8.8±0.2 nS, n=9, p<0.0001).

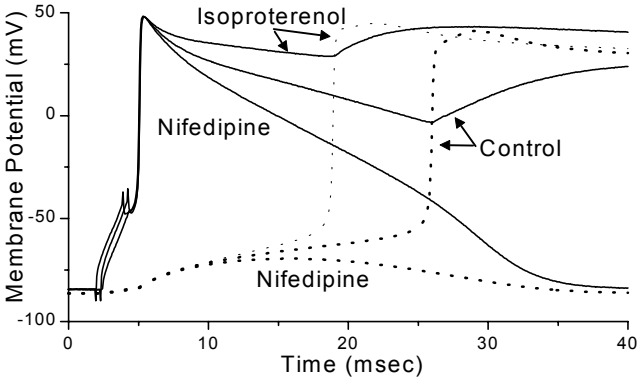

Fig. 2. Action potential transfer between a real guinea pig ventricular cell (leader cell, stimulated at 1 Hz; solid lines) and a model guinea pig ventricular cell (follower cell, not directly stimulated; dotted lines) under control conditions, and upon exposure of the real cell to 20 nM isoproterenol or 1 μM nifedipine. Cells coupled together using the model clamp technique of Fig. 1C with the intercellular coupling conductance G_c set to 6.6 nS.

IV. Concluding Remark

In courses on cardiac electrophysiology, the model clamp system may provide a useful computer tool. Specifically, it can be very effective in gaining insight into the mechanisms of action potential transfer between myocardial cells.

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